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THE FUSION OF VENTRAL CANAL CELL AND EGG IN SPHAGNUM SUBSECUNDUM

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In a previous paper (1) the writer has followed in detail the development of the archegonium of *Sphagnum subsecundum* Nees. At that time the statement was made: "Usually just before fertilization the ventral canal nucleus disintegrates." However, in the early spring of 1917, while attempting to work out the details of fertilization, the interesting fact was uncovered that in the species here studied the ventral canal cell quite often does not disintegrate, but unites with the egg. It seems worth while, therefore, to report the facts in detail.

MATERIAL AND METHODS

The area from which the material came is a grassy bog of about 20 acres near Mineral Springs, Indiana, 40 miles south of Chicago. In the summer and fall of 1912 this bog contained a sufficient amount of water to prevent fires from damaging the polsters of Sphagnum which were scattered throughout the bog. The material is probably dioecious, occurring generally in well defined polsters of one sex or the other. In a few cases mixed polsters were found, but in no instances were the sex organs found together in the same head or upon the same upright branch. The well defined differences in the appearance of male and female plants when the sex organs are approaching or have reached maturity have been stated in the previous paper, but will be repeated here for clearness.

The heads of antheridial plants are decidedly globose and show variations in color from yellow-brown to red-brown and sometimes almost black. Dissection reveals antheridia most of which are apparently at or near maturity. The heads of archegonial branches are less globose and have a somewhat flattened aspect on top. There is no unusual coloring except in the conspicuous bud in the center of the head. This bud varies in color from yellow-brown to red-brown and stands out in sharp contrast to the other portions of the head. An analysis shows archegonia, some young, others almost mature, as terminal structures on short side branches very close to the apex of the main axis, the coloring matter being in the peri-

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chaetial leaves surrounding the organs. These well defined characters made field work a very simple and easy matter once they were determined.

In the latter part of November and during December, 1912, a careful survey of the bog was made, the best polsters being staked out with serially numbered stakes: one series for polsters of plants bearing only archegonia; another for those bearing only antheridia; and a third series for the areas in which the sexes were mixed together, where it was hoped to secure material for a study of fertilization. Notes on field and laboratory observations were kept, and from them the following facts are taken.

By the end of November, 1912, the weather had become very cold, the bog being frozen to the depth of several inches. Blocks of frozen plants together with the frozen mud on which they were growing were cut out with a hatchet and carried in to the laboratory for observation and study. There the blocks of plants were transferred to glass jars containing several inches of water. These jars were kept partially covered with glass plates. A dissection of the material this same evening (November 28) showed the following condition: The dehiscence of several antheridia was observed, the antherozoids being quite active, but most of the antheridia had not reached maturity. Many of the archegonia appeared to be mature, but it was difficult to find one in which the cap had burst and the pathway was open for fertilization. The ventral canal cell and the egg could be easily seen in most of the archegonia. At this time of the year they stand out as two well defined balls of cytoplasm in the center of the archegonium. These rounded protoplasts are frequently so clearly defined in the living material that they can be accurately measured with an ocular micrometer. The same is also true of the nucleus of each protoplast.

For the study of details a considerable amount of material was killed in a fluid made up as follows:

Chromic acid crystals	1 g.
Glacial acetic acid	1 cc.
Water	400 cc.

The following method was employed. Using a pair of forceps with sharp, slender points, the colored buds were snipped quickly and easily out of each head, and were either transferred immediately to the killing fluid, or, if too many sterile branches were included, the latter were cut away in water under a dissecting microscope, using needles for the purpose, and the bud was then put into the killing fluid. The numerous very short side branches bearing archegonia form a firm, compact bud which may be handled by this method without the slightest injury to the archegonia, which latter are well protected by the perichaetial leaves closely investing them.

During the period from December 1 to December 6 there were warm gentle rains. On the 6th a cold wave arrived, again freezing the bog. For the remainder of the month the weather was generally cold and dry with little snow. On December 26 a considerable amount of fresh material

was brought in from the field for further study. In general there was little change to be observed in the sex organs. Very few of the antheridia had dehisced, and only occasionally was an archegonium to be found in which the cap had broken open. But in my notes there appears a fact interesting in the light of subsequent events. A number of cases were observed in which the ventral canal cell had disappeared. On this same evening a large amount of material was killed chiefly in the above mentioned fluid, using the method already described, and it is from this material that the facts here recorded were obtained. The alcohol-xylol method of dehydration and embedding in paraffin was used. The material was cut 5–6 μ in thickness on a rotary microtome. Safranin in combination with Licht Grün, and Heidenhain's iron-alum haematoxylin were used as stains.

It may be of interest to record briefly the further history of the bog and the material. Events were closely followed. The summer of 1913 was hot and very dry. The water level of the bog fell rapidly in the early summer and was never regained. In the spring of 1914 this bog and the country for several miles about were completely burned over by fires which swept the region. The Sphagnum was badly damaged but not entirely destroyed. However, subsequent fires seem to have completed the work of destruction. The writer revisited the area in the early spring of 1917 but was able to find only a few struggling plants where before there had been splendid polsters.

Historical

The appearance of the mature archegonium of Sphagnum seems first to have been described by Hofmeister (3), who represents a transverse wall as separating the ventral canal cell and the egg. The former cell is shown as smaller than the latter, this being especially true in comparing the protoplasts and the nuclei of the cells. The rounding off of the two protoplasts is clearly pictured.

A few years later Schimper (7) describes the "Keimzelle" of the fully developed archegonium as follows: "Diese sah ich bei Sphagnum immer ei- oder umgekehrt birnförmig, im letzteren Falle häufig den oberen engeren Theil von dem unteren weiteren durch eine Querwand gesondert." In his Plate 9, figure 13, he shows an archegonium with the protoplasts of egg and ventral canal cell widely separate. No wall is pictured, though he speaks of it in the text. Attention is called to the fact that the nuclei can be seen through the cells of the venter of the living archegonium. In regard to the "Keimzelle" Schimper says further: "Ich fand selbst Keimzellen, welche an beiden Enden eine Querwand zeigten (fig. 16)." Whether he observed three-celled embryos, or the result of what occasionally occurs in Sphagnum—the subsequent division of either the egg or the ventral canal cell—cannot be stated with certainty. That the three-celled structure is shown as though dissected from the archegonium would lead one to suspect the former case.

In 1872 Roze (6) draws very clearly (Pl. 1, fig. 8) in a mature archegonium of *Sphagnum cymbifolium* the rounded protoplasts of the ventral canal cell and the egg, the latter being pictured as slightly larger than the former. Roze calls the protoplasts "gonosphéries ou globules germinatifs," and refers to the two nuclei as "deux nucléoles primaires." He speaks of the persistence of the two globules (protoplasts) which he says remain up to fertilization, a condition that appears to be peculiar to Sphagnum. He finds the same characteristics in the archegonia of *Sphagnum subsecundum* and *S. acutifolium* as given above for *S. cymbifolium*.

In 1887 Waldner (8), studying the development of the sporophyte of Sphagnum, pictures (Pl. II, fig. 1), according to his explanation of the plates, a longitudinal section of a mature archegonium of *Sphagnum acutifolium* Ehrh. The egg is shown as distinctly egg-shaped, occupies the whole of the venter, and contains a large nucleus with a distinct nucleolus. A fertilized egg is also pictured (Pl. II, fig. 2), but since no adequate description is given of the details one is left in doubt as to the objects figured.

In 1897 Gayet (2) describes the egg of a mature archegonium as a large elliptical cell, being elongated in the direction of the axis of the archegonium. The nucleus is almost spherical and possesses always two nucleoli. The ventral canal cell is described and figured as biconvex.

In 1915 the writer (1, pp. 48, 49) gave the following description of events in the venter of a maturing archegonium: "The ventral canal nucleus produced by this division [*i.e.*, of the ventral cell] is peculiar, being only a trifle smaller than the egg [nucleus]; and is remarkable in that it is regularly persistent and behaves for a time just as does the egg. Not long after the division into ventral canal cell and egg the canal row begins to disintegrate (this process having a variable beginning, though quite often acropetal), but not so the ventral canal cell. Its cytoplasm begins to condense about the nucleus (the same process occurring about the egg), and soon we have in a mature archegonium the appearance of two eggs separated by a wall. Later the cytoplasm about each of these two nuclei becomes markedly condensed and rounded off and may be easily observed in the living material. Still later the wall between the two cells breaks down and the nuclei, each as the center of a ball of cytoplasm, come to lie near together in the venter of the archegonium. . . . Double venters (fig. 42), unequal division of the venter, the ventral canal nucleus larger than the egg (fig. 43), ventral canal nucleus the same size as the egg (fig. 44), and multiple eggs (fig. 45) are not of rare occurrence."

In 1916 Melin (5, pp. 300, 301) says: "Das Resultat [*i.e.*, of the division of the ventral cell] sind zwei Zellen die gewöhnlich ungefähr gleich gross sind. Manchmal kann die obere, die 'Bauchkanalzelle,' etwas kleiner aln die untere, die Eizelle, sein. Beide runden sich bald ab, und wir erhaltes zwei kugelförmige Zellen, die morphologisch so gleichartig sind, dass meiner Ansicht nach kaum ein giltiger Grund besteht, sie mit verschiedenen Namen

zu bezeichnen, weshalb ich sie beide Eizellen nenne. . . . Jede der beiden Eizellen hat einen grossen ziemlich chromatinarmen Kern mit deutlichem Nucleolus; der Kern ist von ungefähr gleichförmigen Plasma umgegeben. Bald verschwindet die Zellwand zwischen ihnen, und sie liegen nun frei in der Bauchhöhle."

The fusion of the ventral canal cell and the egg has been reported but once in the Musci. In 1908 J. and W. Docters van Leeuwen-Reijnvaan (4) published a remarkable article on the sexual process and spermatogenesis in several species of Polytrichum. Briefly stated their results are as follows: In the gametophyte generation there are six chromosomes, which is also the number in the cells of the antheridium. But in the final division in the antheridium a reduction process takes place so that each antherozoid receives three chromosomes. In the archegonium the division of the ventral cell produces a ventral canal cell and an egg which are equal in size. During this division a reduction process is also said to occur so that each of these cells receives three chromosomes. The protoplasts of the ventral canal cell and the egg fuse while the neck of the archegonium is still closed. After the cap breaks open this fusion cell is fertilized by two antherozoids. In this manner the sporophytic chromosome number is restored.

In 1913 Walker (9) published the results of his study on the behavior of the egg and the ventral canal cell in *Polytrichum formosum* and *P. commune.* More than one hundred archegonial rosettes were sectioned but no case of a fusion could be found. Walker thinks the appearance of fusion of the ventral canal cell and egg reported by the van Leeuwen-Reijnvaans is due to their method of fixation.

Development of Ventral Canal Cell and Egg

The ventral cell of *Sphagnum subsecundum* generally divides late into ventral canal cell and egg. The division of cells in the neck is almost if not quite complete when this division occurs. The ventral canal cell is not only persistent but remarkably variable in size. As a very general statement one may say that this cell and its nucleus are a trifle smaller than the egg and the egg nucleus (figs. 2, 8). However, the exceptions are numerous. Often the two are identical in size both as regards the protoplasts and the nuclei (figs. 3, 4), while more rarely the ventral canal cell is larger than the egg in both of these respects (fig. 9).

Shortly after the division of the ventral cell the cells of the canal row begin to disintegrate, but this process has not as yet been found to affect the ventral canal cell. The protoplast of this cell begins to round off, the same process having begun in the egg, the wall between the two cells breaks down, and we have the appearance of two well rounded eggs which soon come more or less in contact in the venter of the archegonium (figs. 3, 8, 9). About this time there may appear, especially in the upper portions of the venter, more or less faintly staining bodies which probably take their origin

from the disintegrated canal cells above. Sometimes these bodies lie close to the ventral canal cell (figs. 3, 8). A rare exception is shown in figure 4 in which a body from the neck has apparently joined itself to the protoplast of the ventral canal cell.

The Fusion of the Protoplasts

As illustrated by figures 4, 5, and 6, the protoplasts unite completely. This fusion is followed later by the union of the nuclei. Unfortunately the killing agent employed, while giving excellent morphological results and little plasmolysis, is not very satisfactory from a cytological standpoint, hence the details of chromatin behavior cannot be accurately reported. In general the chromatin of the two nuclei appears to be more or less intermingled. There is no tendency for each mass to remain distinct.

Every nucleus of *Sphagnum subsecundum* which has been observed thus far, with the exception of that of the fusion cell here described, whether it be of the gametophyte or of the sporophyte generation, is characterized by one conspicuous well-rounded nucleolus. The fertilized egg may prove an exception, since its nucleus has not been seen in a satisfactory preparation. The only other exception is to be found in this fusion nucleus. No case has yet been observed in which the two nucleoli of ventral canal cell and egg have united, though such a condition would seem perfectly possible. In all the cases so far observed the fusion occurs while the neck of the archegonium is still closed. There is, therefore, no danger of mistaking this fusion nucleus for that of a fertilized egg.

The Disintegration of the Ventral Canal Cell

In the material studied, clear cases of the disintegration of the ventral canal cell have been found a number of times. There is no doubt that the ventral canal cell frequently disintegrates; but a summary of the large number of slides studied thus far shows that the number of cases of the union of the two cells about equals the number of cases in which it is certain that the ventral canal cell has disintegrated. In a large number of cases the ventral canal cell was still persistent and was more or less in contact with the egg, as illustrated in figure 3. Two cases were found in which the nucleus of the egg appeared to be degenerating, while that of the ventral canal protoplast just above it was very clear, and sharply defined.

Discussion

It is evident from an examination of the literature quoted that the rounded appearance of the protoplasts of the mature archegonium is not due to the killing agent but is a condition which may be observed and even measured in living material. The average of a number of measurements of the diameters of living protoplasts compared with a like average of killed and stained protoplasts shows that there has been some contraction due to the killing, both in protoplasts and in nuclei, but the contraction is relatively

small and could not bring about the facts which have been observed. Figures 1 and 2 show the exact amount of plasmolysis due to killing and fixing.

Furthermore, I am unable to believe that the technique employed is responsible for the fusions. Using the same methods described in this paper I killed at various times in the fall of 1913 large amounts of Sphagnum for a study of the development of the archegonium. In no cases could be found the slightest trace of injury to the sex organ at any stage of its development. No canal cells were ever observed in the venter, and only after the disintegration of the canal row did any of the contents of the neck begin to make their appearance in the venter. At a later time this disintegrated matter fills the venter with a slimy mucilaginous mass which makes the study of fertilization extremely difficult.

Still more important evidence that the technique is not responsible for the facts is that it is possible to demonstrate *stages* in the fusion of the protoplasts and the nuclei. Not only that, but on a slide from a single head appear archegonia showing the following conditions: (1) Protoplasts of the ventral canal cell and the egg not in contact. (2) Protoplasts have fused, but nuclei, while in contact, are still separate and distinct. (3) Protoplasts and nuclei have fused completely.

It seems hardly reasonable to believe that the technique could bring about the appearance of these varying stages in a single head.

Insofar as the writer is aware, the archegonium of Sphagnum is unique among the Musci in that it comes to maturity in the late fall, withstands the severity of winter, and the egg is fertilized in the early spring. It undergoes great changes in temperature in the alternate freezing and thawing of certain winters; and when snow is absent and the temperature is low it is subject not only to freezing, but no doubt to considerable drying as well. It may be that these severe external conditions furnish the stimulus which brings about the fusion of the protoplasts.

As yet I am unable to make any statement in regard to the behavior of the fusion nucleus. Whether it may develop directly into a sporophyte, whether or not it is capable of being fertilized, and whether or not this fusion is peculiar only to the species here studied—all these questions must await further work.

Summary

1. The ventral canal cell of *Sphagnum subsecundum* is regularly persistent, and variable in size.

2. The protoplasts of ventral canal cell and egg round off and, the wall between the two disintegrating, they lie near together in the venter of the archegonium.

3. In material killed in the latter part of December a number of cases of the fusion of these protoplasts have been found.

4. The fusion of the protoplasts is followed by the fusion of the nuclei.

5. Undoubted cases of the degeneration of the ventral canal cell have also been found.

6. Occasionally the egg may degenerate, while the protoplast of the ventral canal cell remains functional.

7. Further work is needed to determine how general this condition is in other species of Sphagnum, and to follow the history of the fusion nucleus.

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EXPLANATION OF PLATES

All figures were drawn at table level with the aid of a camera lucida, using Spencer ocular 10 x and 1.5 mm. oil immersion objective. Being reduced one half in reproduction, they show a magnification of approximately 1000 ×.

PLATE XIV

FIG. 1. Ventral cell of an archegonium having eight neck canal cells. Peculiarly shaped plastids in the cytoplasm.

FIG. 2. Ventral canal cell and egg still separated by a wall, showing the difference in size of the two nuclei. Contraction of the protoplasts is probably due in large part to plasmolysis.

FIG. 3. Wall has disintegrated, protoplasts have rounded off and are lying in contact in the venter. Nuclei and protoplasts practically identical in size.

FIG. 4. The two protoplasts in closer contact but outline of each distinct. Disintegrated material from canal row in contact with upper protoplast.

FIG. 5. Protoplasts have completely fused. Nuclei in contact, but each distinct.

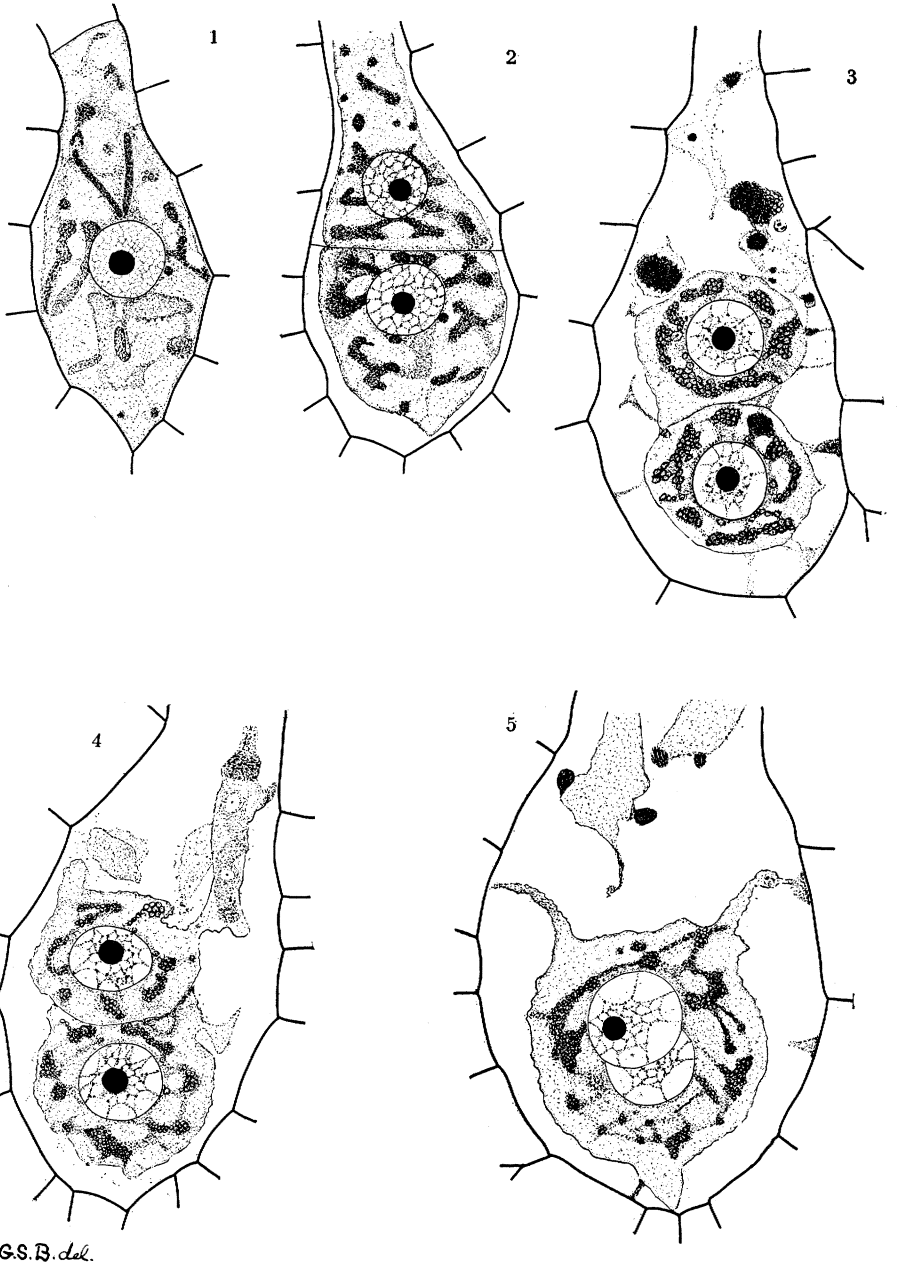
PLATE XV

FIG. 6. The fusion of protoplasts and nuclei completed. Mucilaginous matter beginning to appear about the protoplast.

FIG. 7. The disintegration of the ventral canal cell. Only a blurred mass remains.

FIG. 8. The rounded protoplasts. That of the ventral canal cell is smaller and has a smaller nucleus than the egg.

FIG. 9. Protoplast of the ventral canal cell is larger and has a larger nucleus than the egg.



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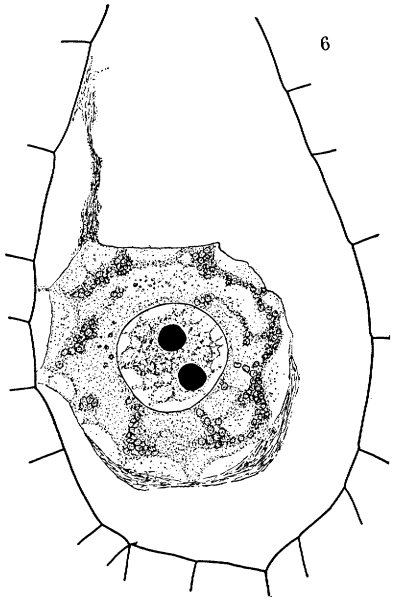

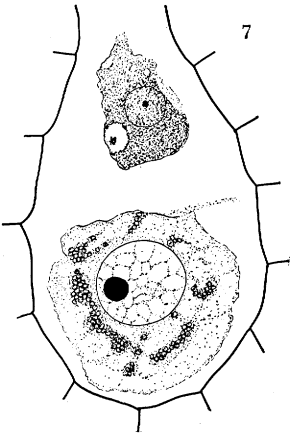

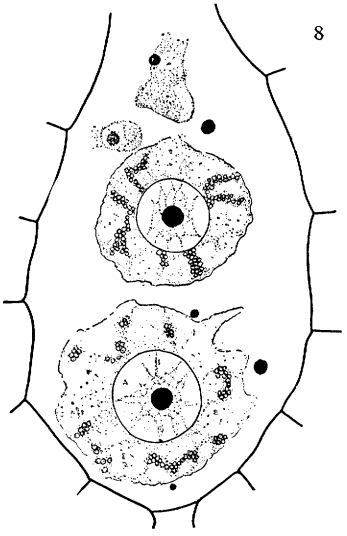

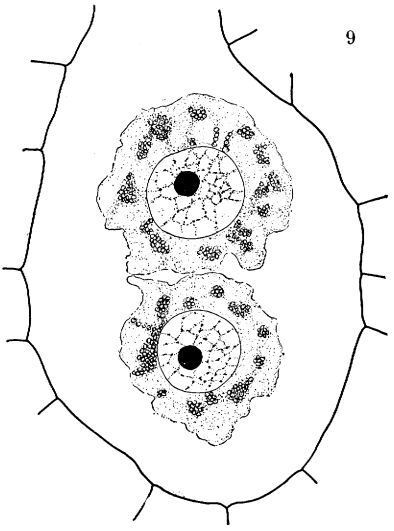

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